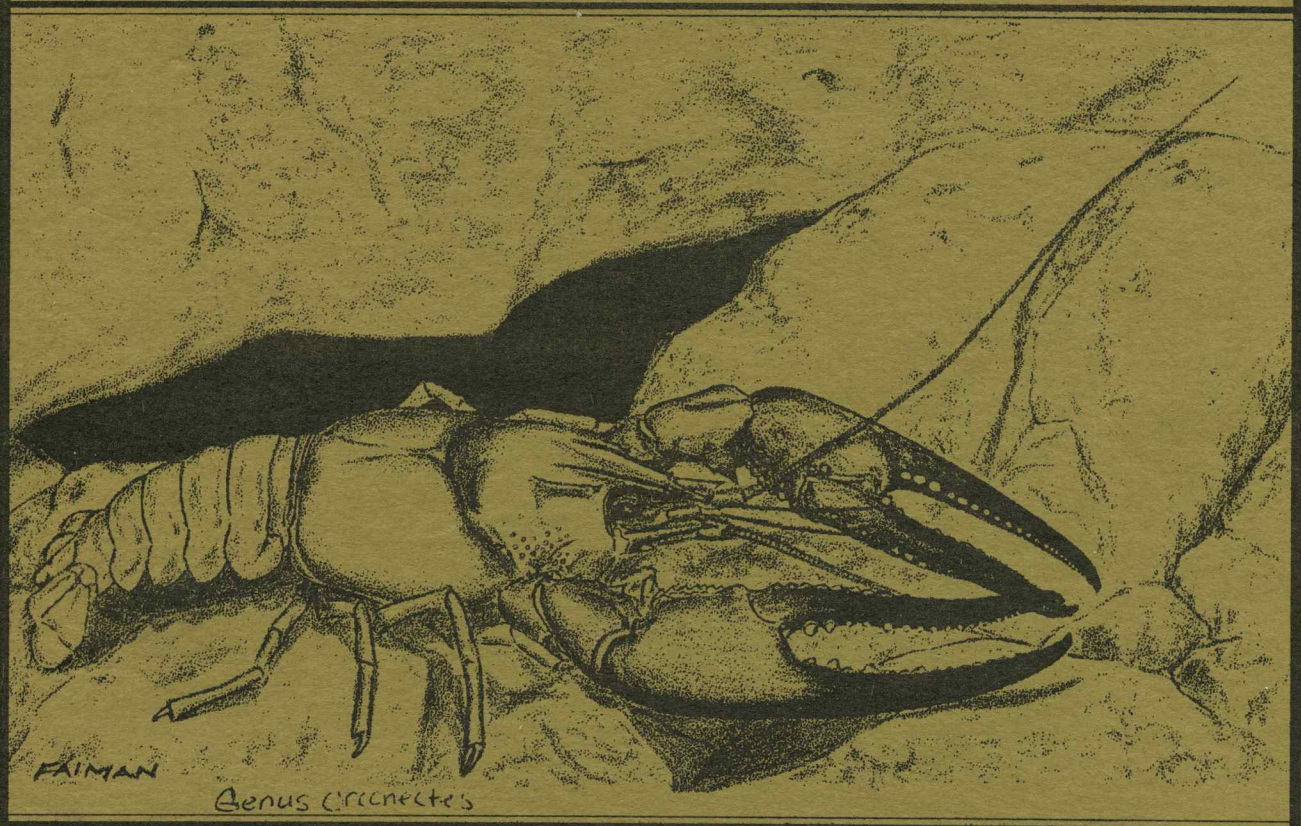
Development of a Quantitative Sampling Method to Assess Crayfish Communities and Macrohabitat Associations in Missouri Ozarks Streams



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FINAL REPORT

DEVELOPMENT OF A QUANTITATIVE SAMPLING METHOD TO ASSESS CRAYFISH COMMUNITIES AND MACROHABITAT ASSOCIATIONS IN MISSOURI OZARKS STREAMS

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Abstract

Aquatic biologists depend on the ability to perform quantitative assessments of the populations and communities they study and manage. There has been limited work done to develop and evaluate the reliability of such methods for sampling stream-dwelling crayfishes. We desired a quantitative sampling method to assess crayfish in Ozarks streams. Our objectives were to 1) develop a method to obtain and compare representative density estimates with acceptable variance and reasonable sampling effort, 2) describe diurnal habitat associations by stream crayfish communities, and 3) develop and evaluate a practical relative density method for stream managers to routinely monitor crayfish densities, size structure, and noteworthy density fluctuations. We evaluated two gears; baited traps and a 1-m^2 quadrat sampler. Traps were tested only briefly and found to be unacceptable because early results were highly variable, biased for sex and size, and included many empty traps. The quadrat sampling method used a two-step stratification procedure that stratified sampling effort among five macrohabitats to reduce variability. Results indicated generally high diurnal mean crayfish densities, and the method performed well during summer and fall, detecting spatial differences among macrohabitats and temporal differences among years. Sampling precision, as measured by coefficients of variation was acceptable but not high. Statistical power was generally good for detecting spatial differences, but reduced and variable for detecting temporal changes. Macrohabitats with slower current velocities showed the highest densities, and results suggested macrohabitat selectivity by the crayfish community. The relative density method yielded unacceptable results, but could be significantly improved with increased sampling effort to produce an acceptable monitoring tool, depending on objectives.

Introduction

Crayfish are widely recognized as an important ecological component of stream systems. They function in the processing of organic matter, and the transformation of energy between trophic levels and the structuring of benthic invertebrate communities (Lorman and Magnuson 1978, Momot et al. 1978, Creed 1994, Momot 1995, Rabeni et al. 1995, Parkyn et al. 1997, Whitledge and Rabeni 1997, Parkyn et al. in press). Crayfish are also a major food source for a variety of animals including important sport fishes (Rabeni 1992, DiStefano 1993, Roell and Orth 1993) . In 1990, we initiated a long term project to learn about Missouri Ozarks stream crayfish population dynamics, life histories, habitat requirements, their significance as prey for sport fishes, and their response to specific fisheries managment practices. To meet these objectives we desired a quantitative sampling method that would allow us to assess crayfish populations and communities in Ozarks streams.

Aquatic biologists frequently depend on the ability to estimate densities, abundance or relative abundance of the populations and communities they study and manage. Benthic macroinvertebrates such as crayfish typically show spatial heterogeneity or clustered distributions due to several factors, including habitat preferences (Skurdal et al. 1988, Rabeni 1985), predator-influenced behavior (Stein and Magnuson 1976, Collins et al. 1983), and changing environmental conditions such as temperature (Somers and Green 1993). Clustered distribution patterns present sampling problems partly because large sample sizes are required to estimate population densities with acceptable precision (Thompson et al. 1992).

The primary purpose of this study was to develop a quantitative method to obtain and compare, spatially and temporally, representative density estimates of stream crayfish communities with an acceptable level of among-sample variance and requiring reasonable sampling effort. Our

second objective was to examine general patterns of diurnal habitat associations by Ozarks stream crayfish communities. Third, we sought an additional practical method (less labor intensive) that managers might use to routinely assess relative density, size structure, and noteworthy crayfish community fluctuations in Ozarks streams.

A variety of gear types and methods have been used to sample crayfish, but the development of an efficient, reliable method to obtain population or community estimates in streams has been hindered because most gears and methods select for specific life stages, sexes, or species (DiStefano 1993, Rabeni et al. 1997). We chose to evaluate two gear types; a 1-m^2 quadrat sampler (Rabeni 1985) for precise density and relative density estimates, and baited, wire funnel traps (Capelli and Magnuson 1983) for relative density estimates. The precise density estimation method we tested, using the quadrat sampler, was designed to address clustered distributions of crayfishes over a variety of macrohabitat types and the differential use of macrohabitats by various species and life history stages. We evaluated the relative density method for managers in terms of effort required, as well as ability to assess relative density and size structure. Our evaluation of baited traps was less intensive. We determined during a pilot study that they might not suit our needs, but we collected additional data to support or refute our suspicions.

Study Sites

Our study was conducted on two Ozarks streams, Jacks Fork River and Big Piney River. Jacks Fork River is a sixth order, easterly-flowing cool-water tributary to Current River in southern Missouri, with a mean annual discharge of 12.5 $m^3 \cdot s^{-1}$ (Rabeni 1992). A near-pristine stream, it flows mostly through the Ozark National Scenic Riverways, managed by the U. S.

National Park Service. Four species of crayfish occur in Jacks Fork River, but only three were commonly captured; *Orconectes luteus*, *O. ozarkae* and *O. punctimanus*. Big Piney River is a sixth order tributary of the Gasconade River that originates in the Ozarks highlands of south-central Missouri and flows north, much of it through pasture land. It has a mean annual discharge of 15.4 $m^3 \cdot s^{-1}$ (Reed et al. 1994). Big Piney River harbors *O. luteus* and *O. punctimanus*. Although these rivers have many differences, both are moderately productive (Rabeni et al. 1995), spring-fed, contain well-developed riffle and pool sequences, and patches of emergent vegetation, primarily water willow (*Justicia* sp.), are common along shallow margins during the summer. We used two study sites on each river for fall sampling and only one site per river for summer sampling. Jacks Fork River sites were both near Mountain View, Missouri; site 1 was at Ratcliff Ford, approximately 1.3 km upstream of Highway 17 bridge, and site 2 at Blue Spring, about 4.5 km downstream of Highway 17 bridge. Big Piney River sites were both near Houston, Missouri; site 1 was 0.2 km downstream of Mineral Spring Access, and site 2 was 1.0 km downstream of Sand Shoals bridge. All sites were between 0.8 and 1.3 km in length, and each site contained at least two major pools, three riffles and assorted runs, emergent vegetation patches, and backwater/forewater pools. Jacks Fork River sites had a gradient of about 1.78 $m \cdot km^{-1}$, averaged 0.6 m in depth and 20 to 25 m in width; Big Piney River sites dropped 0.77 $m \cdot km^{-1}$, averaged 0.5 m in depth and 18 to 25 m in width. A general description of water chemistry for these streams is provided in Table 1.

Methods

Trapping

We evaluated commercially manufactured, baited traps (Gee's hardware cloth minnow

traps) in Jacks Fork River during the summers of 1991, 1993, 1995 and 1996 (total of 11 nights, n = 187 traps). Standard traps had 6-mm mesh and two funneled entrances measuring 25-mm diameter, but we also evaluated effects of larger (modified to 38 mm) entrances (Collins et al. 1983, Somers and Green 1993). Traps were baited with 15 to 20 g of commercial, canned, liver-flavored dog food enclosed in a perforated, plastic, 34-mL container (film canister). Traps were weighted, marked with a tethered float, and placed randomly, but at least 10 m apart on the substrate throughout pools. We set 10 to 30 traps between 1600 h and 1800 h and retrieved them the following morning (0800 h to 0900 h) (Somers and Green 1993). Trap catches were recorded and the following parameters were determined or measured: species, sex, reproductive form (form I = reproductively active, form II = reproductively inactive or immature), carapace length (CL, from tip of rostrum to posterior edge of the cephalothorax) to the nearest 0.1 mm, and wet weight (g). We noted recently molted specimens and females carrying eggs (ovigerous) or hatchlings.

We compared differences in mean catch per unit effort (CPUE, number of crayfish • $trap^{-1}$ • $night^{-1}$) and mean CL using approximate 95 % confidence intervals of the form $\theta \pm 2SE(\theta)$ (Cochran 1977).

Quadrat sampling

Quadrat sampling method. -The 1-m^2 quadrat sampler we evaluated was constructed with a 12-mm angle-iron frame, standing 0.51 m high, and covered on three sides with 2-mm by 3-mm rectangular-mesh netting (Figure 1). We attached a 1.22-m-long bag made from the same netting on the fourth side (downstream side).

We collected 111 samples from rivers being considered for study during a pilot study in

1990 to provide preliminary estimates of sampling variance (Morin 1985). Based on the pilot sampling and sample size determination procedures (Cochran 1977, Elliot 1979), we designed a two-step sampling procedure to minimize variability and increase our ability to estimate crayfish densities with a reasonable amount of effort. In the first step we partitioned a stream into "primary" and "secondary" crayfish habitat based on surface substrate composition. The second step involved partitioning the stream into and stratifying the sampling effort among common macrohabitat types as suggested by Roell and Orth (1992).

We classified secondary habitat as stream areas where silt, sand, fine gravel, or bedrock were the predominant substrate, because we observed that crayfish were scarce and that sampling these areas was unproductive and greatly increased among-sample variance. In contrast, crayfish appeared concentrated in primary habitat, dominated by substrate > 16 mm diameter (pebble, cobble, boulder; according to a modified Wentworth scale; Bovee and Milhous 1978). We restricted our sampling to primary habitat by placing our quadrat only in areas of the stream where a square meter of substrate contained at least 1% pebble (16 - 65 mm diameter), cobble (65 - 250 mm diameter) or boulder (>250 mm diameter). Thus, we eliminated areas where the substrate was comprised totally of silt (0.063 mm diameter), sand (0.06 - 2 mm diameter), gravel (2 - 16 mm diameter), and bedrock. Focusing our effort on habitats that yield the largest catches introduced subjectivity, but this strategy has been used in previous crayfish studies to produce reliable results (France et al. 1991, Somers and Green 1993). We believe this was warranted because it greatly reduced among-sample variance that would have been associated with a more random sampling design, and it greatly reduced the effort required to obtain reliable quantitative estimates. In addition, intensive mapping of our study sites revealed that secondary habitat composed < 6 % of the substrate area in our streams.

Early in the study we collected data to support our decision to eliminate secondary habitat from our long term sampling program. We collected 83 quadrat samples (65 in June, 18 in August) in typical secondary habitats in Jacks Fork and Big Piney rivers to determine mean crayfish densities.

We found primary and secondary habitats easy to distinguish in the field; however, to add support to our subjective classification we chose to quantify this using a discriminant function analysis (DFA). During each quadrat sample, two individuals independently assessed the surface substrate composition inside the 1-m^2 quadrat. They estimated the relative proportion (%) of eight substrate categories (bedrock, silt, sand, gravel, pebble, cobble, boulder, and detritus [dead and decaying organic matter]) using reference tools (steel disks cut to appropriate sizes) and recorded the mean of the two estimates for each category (n = 1142 Jacks Fork River primary habitat samples, n = 37 Jacks Fork River secondary habitat samples, n = 819 Big Piney River primary habitat samples, n = 46 Big Piney River secondary habitat samples). We developed two variables from substrate estimate data for the DFA: BOCOPEB was the sum of the proportions (%) of boulder, cobble, and pebble in each sample; SANGRBED was the sum of the proportions (%) of sand, gravel, and bedrock in each sample. These variables were transformed to meet assumptions associated with DFA (Krzanowski 1977), creating two new variables,

$$\text{logbcp} = \log_e\left(\frac{\text{BOCOPEB}}{1 - \text{BOCOPEB}}\right) \quad \text{and} \quad \text{logsgb} = \log_e\left(\frac{\text{SANGRBED}}{1 - \text{SANGRBED}}\right).$$

Then we constucted discriminant functions for both Jacks Fork and Big Piney rivers, and these were used to construct classification rules. The effectiveness of a discriminant function classification rule was assessed by misclassification rates. A misclassification occurred when an

individual secondary habitat sample was misclassified as primary habitat or vice-versa in our field sampling as compared to the entire database. This provided an estimate of our ability to correctly classify primary and secondary habitat in the field and supported our subjective procedure. In addition, the DFA quantitatively defined primary and secondary habitat, serving as a guide in future sampling.

In the second step of our sampling procedure, we eliminated all secondary habitat from consideration, and we stratified our effort in terms of number of quadrat samples among five macrohabitats: riffle, run, pool, back/forewater (or side channel) pool, and emergent aquatic vegetation patch (water willow). These macrohabitats are not completely discrete and some overlap exists (Jowett 1993), but we were confident in delineating them in most instances, and current velocity and depth measurements helped confirm these beliefs. Riffles were shallow (95 % confidence interval for both rivers = 0.17 - 0.22 m-depth), fast water (0.14 - 0.24 m • s^{-1}, current velocity at 2 cm above substrate [CV]) habitats with notable surface turbulence. Runs were of moderate depth (0.32 - 0.40 m), with slow to moderate CV (0.06 - 0.12 m • s^{-1}) and minimal surface turbulence. Pools were deep (0.40 - 1.16 m) with minimal CV (0.02 - 0.04 m • s^{-1}). Back/forewater pools (hereafter referred to as backwaters) were generally shallow (0.18 - 0.31 m), had minimal CV (0.00 - 0.01 m • s^{-1}), were partially isolated from the main channel, and often ephemeral. Emergent aquatic vegetation patches (hereafter referred to as vegetation patches) were shallow (0.16 - 0.23 m), had minimal CV (0.01 - 0.04 m • s^{-1}), and typically associated with stream margins and gravel bars.

We chose a stratified quadrat sampling allocation strategy according to formulas detailed by Scheaffer et al. (page 109, 1990) to estimate mean crayfish densities within a prespecified bound for error, while minimizing sampling effort at each site. This strategy was selected because

it allocated our sampling effort based on three important factors: 1) variation (standard deviation) in mean crayfish densities per strata (macrohabitat), 2) the proportional geographic area of each strata composing the study site, and 3) the cost (time required) of obtaining a sample in each strata. Analysis of the pilot study quadrat data provided mean crayfish densities for each strata and allowed us to determine that it was desirable to set the error bound within about 20 % of the overall mean crayfish density. Each study site was intensively mapped to provide proportional area for each strata. The cost for sampling each strata was estimated by ratios; riffles, runs and backwaters required approximately equal effort and were assigned a cost of 1.0. Vegetation patch samples required more time and were assigned a cost value of 1.5. Pool samples generally required the most time (due to depth) and were assigned a cost value of 2.0. Costs were assumed to be equal throughout the study.

Logistics, time constraints, and number of study sites largely determined the maximum sampling effort (number of quadrat samples) we could expend at each site during a season. Based on experience from the pilot study we determined that we could obtain a maximum of 240 - 250 total quadrat samples per season to be divided among 4 study sites (60 - 63 samples per site). Allocation of those 60 - 63 quadrat samples among our 5 macrohabitats (strata) at each site was estimated by:

$$n = \frac{\left(\sum_{i=1}^{5} \frac{a_i s_i}{\sqrt{c_i}}\right)\left(\sum_{i=1}^{5} a_i s_i \sqrt{c_i}\right)}{\frac{B^2}{4}\left(\sum_{i=1}^{5} a_i\right)^2 + \sum_{i=1}^{5} a_i s_i^2}$$

where a_i is geographical area in strata i, s_i is the standard deviation for strata i, c_i is the cost for strata i, B is the error bound that ranges from 1 to 10, and i represents the five different strata (Scheaffer et al. 1990).

Temporal considerations. -We wished to determine appropriate times to quantitatively sample these crayfish communities. Initial observation proved that crayfish in our study streams were typically nocturnal, seeking shelter in the substrate and rarely visible during the day. This enabled us to sample during the day with little concern for crayfish avoiding the quadrat sampler.

We began the study with intensive sampling periods in late spring (late May/early June) and early fall (late August/early September) to examine sampling differences between these seasons. After three years (1991-1993), it became obvious that late spring sampling was unreliable for obtaining density estimates due to greatly increased variability associated with recruitment of young. Young-of-year made up a substantial portion of the community, but in some years were not yet recruited to our sampling gear due to interannual variation in the timing of the spring hatch. In 1994, we eliminated spring sampling in favor of an intensive mid-summer (July/early August) sample. We continued with a less intensive early fall (September) sampling period. Subsequent analysis confirmed that mean crayfish densities for the spring sampling period had much higher overall coefficients of variation (105 % for Jack Fork River, 93 % for Big Piney River) than fall (70 % for Jacks Fork River, 85 % for Big Piney River) or mid-summer (82 % for Jacks Fork River, 82 % for Big Piney River) sampling periods.

Quadrat sampling procedure. -We sampled by placing the quadrat in primary habitat within a designated macrohabitat and sealing it into the substrate to prevent crayfish escape. We

thoroughly disturbed the substrate within the sampler using hand-held garden rakes and feet for 3 to 5 min, until we penetrated the substrate to a depth of at least 15 cm. Crayfish were swept downstream by hand and into the bag. The quadrat was transported to shore where we removed, identified and measured all crayfish as previously described. We used SCUBA in deep water (>1.5 m) and snorkeling gear in depths of 0.5 m - 1.5 m. In a typical day, we obtained 12 to 20 samples, depending upon the crew size, macrohabitats sampled and water depths.

Analysis of quadrat sampling data. -We analyzed all crayfish mean density data from quantitative quadrat sampling with either two-way analysis of variance (ANOVA) or three-way ANOVA to test for differences in macrohabitat use by the crayfish community (species combined). Two-way ANOVAs were used for summer data from both rivers (rivers analyzed separately, only one site per river) where mean density was the dependent variable, and independent variables were macrohabitat and year. Three-way ANOVAs were used for fall data from both rivers (two sites per river) where mean density was the dependent variable, and independent variables were macrohabitat, year and site. All data were log transformed to meet normality assumptions. When significant differences were detected by ANOVA, we used a Least Squares Means Probability Difference Analysis (LSMPDA) to determine where differences occurred (SAS Institute Inc. 1989) All analyses used an alpha level of $P = 0.05$.

Edgewater sampling. -We observed during the first year of the study young-of-year crayfish congregating in shallow, near-shore areas of runs and pools in both rivers, to a greater degree than initially expected. We considered designating "edgewaters" as a sixth macrohabitat, but decided against it because the study was underway. Alternatively, for the remainder of the

study, we collected a small subset of our run and pool quadrat samples within 1 m of the shore; these were recorded as edgewater samples. These data were not incorporated into our inferential statistical analyses because they were added after the study was ongoing. They were analyzed descriptively, using simple comparisons of mean crayfish densities (and 95 % confidence intervals) in edgewater samples relative to mean crayfish densities in all other run and pool samples combined.

Relative density method. -We evaluated a less intensive sampling method, using the 1-m^2 quadrat, primarily for estimating relative crayfish density of communities in Missouri Ozarks streams. Secondarily, we explored the potential for assessing size structure of these communities using CL frequency data. We desired a method that could be used by fisheries managers to routinely monitor communities and detect noteworthy temporal changes. Essentially, this method would involve restricting quadrat sampling to a small number of easily accessible riffle and run macrohabitats to minimize sampling effort and expenses (e.g. snorkeling and SCUBA gear).

Our evaluation involved a three-phase approach. In the first and second phases we used a sample size (number of quadrats for density estimates, and number of *O. luteus* crayfish for representative size structure estimates) determination procedure to determine when taking additional samples would not significantly increase precision of the data, but would fit into the constraints determined in the third phase. The third phase involved a simple logistical analysis to determine reasonable expectations for the amount of sampling that might be achieved in a full day by a fisheries management crew.

First, we conducted an intensive evaluation at an extended riffle/run combination reach at our Jacks Fork River site 1 on July 21, 1993. We blocked off an area 15 m in length and 10 m

wide (150 m^2). Prior to sampling, we randomly selected 24 1-m^2 sample plots within the area. Samples were taken and crayfish processed as previously described. Density and CL frequency data were manipulated using a bootstrapping technique in which *i* observations (between 2 - 100 quadrat samples for density data; 50 - 10,000 *O. luteus* CLs for size structure data) were randomly selected (with replacement) to create a new data set termed the sample data. From the sample data set, crayfish density estimates or size structures were generated for the crayfish community and also individually for the predominant species, *O. luteus* (for the size structure analysis, only *O. luteus* data were used), and compared to the estimates generated from the original 24-sample data set (the population data). This procedure was repeated 100 times for each value of *i*, and the squared difference (squared to avoid negative values) between the sample density or size structure and the population density or size structure was calculated each time. Running the program 100 times facilitated determination of the magnitude of error expected when taking *i* samples. From this distribution of errors at each value of *i*, we produced the statistics, mean squared difference and corresponding confidence intervals. By progressively increasing the number of observations selected (i.e. increasing the sample size), we attempted to determine the point when taking an additional quadrat sample contributed little to the overall precision of the density or size structure estimate. For CL frequency data, we also explored the effect of different CL categories (1mm, 2mm, and 3mm) on sample size determination.

In the second phase of evaluating a relative density method, we established a population data set using all riffle and run samples (N = 959 quadrat samples) obtained during Jacks Fork River quantitative crayfish sampling during 1991 through 1998. The bootstrapping technique was used to randomly sample these data as previously described.

Management staff from two Missouri Department of Conservation (MDC) regions

participated in the third phase of an evaluation of the relative density method to supplement the sample size determination efforts. Each region's role was to obtain 15 to 20 quadrat samples per day for 2 days each fall for 5 years, for a total of 20 days of effort and between 300 and 400 samples . This effort was to provide information about feasibility and relative effort required of the method for management crews.

Power analysis. -We performed a power analysis on data collected via our quantitative quadrat sampling to estimate the ability of this sampling strategy and our method to detect significant differences in crayfish densities primarily among macrohabitats (multiple-mean comparisons) within a given sampling period. Also, we determined power to detect temporal density differences among 3- and 5-year sampling periods. Our main concern was Type II error, the likelihood of not finding a significant difference in macrohabitat use when, in fact, a difference might exist. In addition, we used power analysis to assess the ability of our relative density method to detect significant differences in mean crayfish densities within riffle and run macrohabitats at a given site over time (years). We used two-mean comparisons to determine power in ability to detect differences between years, and multiple-mean comparisons for evaluations of differing sample sizes (8 and 14 quadrat samples within riffle and run macrohabitats) over a 5-year period. In the power analysis of differing sample size, we used bootstrapping (as previously described) to randomly select multiple 8- and 14-quadrat riffle or run samples from our entire quadrat sampling database (N = 959). Power was calculated for these multiple-mean and two-mean comparisons according to Somers (1997). For both analyses we used data collected at Jacks Fork River during summer sampling periods from 1994 through 1998, and all data were log-normalized.

Results

Trapping

Baited trap CPUE in Jacks Fork River was highly variable, and traps exhibited biases associated with crayfish size and species. Preliminary data analysis identified important effects of trap entry hole diameter on CPUE and crayfish CL, but results of these analyses supported the pooling of data across seasons and years.

The large number of empty traps (85 of 187, 45 %) contributed to high variability in our trapping data, and to our dissatisfaction with this sampling gear. A normal approximation for a test of differences between two proportions indicated that traps with standard entry holes of 25 mm (49 % empty) were more likely ($P < 0.05$) to be empty than traps with enlarged (38 mm) entry holes (39 %). Modified traps ($\bar{X} = 3.18$, SE = 0.22) also had a greater CPUE ($P < 0.05$) than standard traps ($\bar{X} = 1.37$, SE = 0.64).

Traps appeared to be biased toward larger or older crayfish; 368 adults compared to 2 young-of-year captured, . Modified traps ($\bar{X}$ CL = 43.5, SE = 0.34) caught larger crayfish ($P <$ 0.05) than standard traps ($\bar{X}$ CL = 37.1, SE = 0.53).

Trap catches were dominated by *O. punctimanus* (81 %; n = 300 individuals), followed by *O. ozarkae* (15 %, n = 56), and *O. luteus* (2.7 %, n = 10).

Quadrat sampling

Quantitative sampling method. -Examination of crayfish use of secondary habitats in Jacks Fork and Big Piney rivers indicated that mean crayfish densities were < 1 crayfish • m^{-2} ($\bar{X} = 0.9$ SE $\pm$ 0.2, n = 65 samples in June and n = 18 samples in August), and crayfish were absent from

67 % of these samples.

The DFA performed to quantify our distinction between primary and secondary habitat yielded discriminant functions for the Jacks Fork River sites (logsgb = [0.4 * logbcp] - 0.6) and the Big Piney River sites (logsgb = logbcp + 1). These functions were used to construct habitat classification rules. For Jacks Fork River, habitat was classified as:

secondary if logsgb > (0.4 * logbcp) - 0.6 or BOCOPEB = 0, and

primary if logsgb $\leq$ (0.4 * logbcp) - 0.6 or SANGRBED = 0.

For Big Piney River, habitat was classified as:

secondary if logsgb > logbcp + 1 or BOCOPEB = 0, and

primary if logsgb $\leq$ logbcp + 1 or SANGRBED = 0.

This quantification of primary and secondary habitats using DFA validated our first level of stratification in field sampling. The Jacks Fork River classification rule classified 97 % (36 of 37) of our secondary habitat samples correctly, and 94 % (1077 of 1142) of our primary habitat samples correctly. The Big Piney River classification rule classified 96 % (44 of 46) of our secondary habitat samples correctly, and 98 % (803 of 819) of our primary habitat samples correctly.

Crayfish macrohabitat associations. -Results of quantitative quadrat sampling indicated that our sampling strategy and overall method enabled us to detect spatial and temporal differences among diurnal mean crayfish densities, expressed as number • m^{-2}. Crayfish densities were generally high and these communities were using many of the available habitats within our study streams. Our ANOVAs and LSMPDAs indicated mean crayfish densities differed spatially among

the five macrohabitats across all years during summer in both rivers and during fall in Jacks Fork River (Table 2), and differed spatially between the two sites at Big Piney River ($P < 0.0001$). Densities also differed temporally within macrohabitats ($P < 0.05$) among years at both rivers during both seasons. Significant two-way and three-way interactions occurred in the ANOVAs for Jacks Fork River summer data and Big Piney River fall data, but they were ordered, indicating that no crossover occurred when least square means were plotted, and they masked no main effects (Table 2, Ott 1988).

During summer at Jacks Fork River, the slower velocity macrohabitats, backwaters, vegetation patches and pools, yielded significantly higher mean crayfish (*O. luteus, O. ozarkae* and *O. punctimanus* combined) densities (about 30 • m^{-2}) than runs and riffles across all years (Figure 2). Densities in riffles (13 • m^{-2}) were significantly lower than those in all other macrohabitats. Coefficients of variation among the five macrohabitats for each year (1994 through 1998) during summer at Jacks Fork River (n = 25) ranged from 31 to 90 %, with a mean of 60 %.

A similar pattern was observed during fall at Jacks Fork River (Figure 3). Vegetation patches (42 • m^{-2}) yielded significantly higher densities than the faster velocity runs (22 • m^{-2}) and riffles (17 • m^{-2}) across all years. Riffle densities were lower than all other macrohabitats. Coefficients of variation ranged from 9 to 78 % with a mean of 44 % (n = 30).

During summer at Big Piney River, the slower velocity, shallower macrohabitats, backwaters (52 • m^{-2}) and vegetation patches (44 • m^{-2}), produced significantly higher densities of the two crayfishes (*O. luteus* and *O. punctimanus* combined) than all other macrohabitats (Figure 4). Densities in pools (16 • m^{-2}) were higher than in riffles (13 • m^{-2}). Coefficients of variation ranged from 31 to 99 % with a mean of 60 % (n = 25).

Mean crayfish density patterns among macrohabitats for Big Piney River fall sampling

appeared similar to summer data; vegetation patches (27 • m^{-2}) and backwaters (26 • m^{-2}) harbored high densities relative to the other three macrohabitats (10 to 14 • m^{-2}, Figure 5). However, a lack of samples from vegetation patches and backwaters in fall of 1993 (due to floods) precluded inclusion of those macrohabitats in the corresponding ANOVA, and masked possible differences among all macrohabitats. Coefficients of variation ranged from 31 to 105 % with a mean of 64 % (n = 12).

Power analysis. -Results of multiple-mean power analysis indicated that our quantitative quadrat sampling method had good power to detect spatial crayfish density differences among macrohabitats, but less power to detect temporal (among year) differences within macrohabitats. Power to detect density differences among macrohabitats was 78 % or greater in 4 of the 5 years tested (Table 3, bottom row). Power to detect density differences among years was generally variable, but it was best in run and pool macrohabitats (Table 3, far right column) where ANOVAs indicated that mean densities were significantly different among years (Table 3, second column from right). Power was higher when 5 years of data were analyzed (Table 3, far right column) than when only 3 years of data were used (Table 4, far right column). Power was highest among groups of mean densities that were the most variable (i.e. had the greatest "effect size").

Edgewater sampling. -Results of quadrat sampling in edgewaters at both rivers during two seasons were inconclusive. There appeared to be a general pattern of higher mean crayfish densities in edgewater samples relative to corresponding run and pool samples, but overlapping 95 % confidence intervals suggested that either there were no density differences or that sample variance was too high to detect differences (Figure 6).

Relative density method. -Our evaluations of a potential method using only riffle and run macrohabitats showed mediocre results with regard to estimating crayfish relative densities. The bootstrapping analysis showed that mean squared difference nearly always decreased with an increased number of quadrat samples in both analyses (density and size structure). Bootstrapping employed in the 24-sample intensive evaluation conducted in July 1993 indicated that about 10 (± 2) 1-m^2 quadrat samples were required to produce precise density estimates for all three crayfish species combined (the community) and for *O. luteus* individually (Figure 7). This estimate was derived from examining the percent decrease in mean squared difference resulting from taking additional samples. The data for the crayfish community indicated a 34.6 % decrease in mean squared difference in taking 8 rather than 6 samples, but only a subsequent decrease of 8.6 %, taking 10 samples. Similarly, the data for *O. luteus* indicated a 39.5 % decrease in mean squared difference moving from 6 to 8 samples. There was a 18.3 % drop from 10 to 12 samples (Figure 7).

Repeating the bootstrapping technique on 959 quadrat samples collected in riffles and runs during 1991 through 1998 at Jacks Fork River yielded results similar to the 24-sample intensive evaluation. Data for the community and for *O. luteus* alone indicated that the rate of increased precision due to increased number of samples slowed at about 8 to 12 samples (Figure 8).

Bootstrapping showed less than desirable results for estimating crayfish community or population size structure at the level of effort we used. The 24-sample intensive evaluation conducted in July 1993 indicated that a sampling of about 400 (± 100) or 500 (± 100) members of the crayfish community or members of the *O. luteus* population, respectively, were required to produce precise size structure estimates when 1-mm carapace length increments were used, although there were no definite inflection points in the relationships between mean squared

difference and number of crayfish measured (Figure 9).

Repeating the bootstrapping technique on 959 quadrat samples collected in riffles and runs during 1991 through 1998 at Jacks Fork River yielded results quite different from the 24-sample intensive evaluation. These data indicated that the rate of increased precision in size structure estimates (using 1-mm increments) due to increased number of crayfish carapace lengths measured slowed at about 3000 (± 1000) or 4000 (± 1000) individuals for the crayfish community or for only the *O. luteus* population, respectively (Figure 10).

Results of our analysis to determine the effects of increasing CL increment (1-, 2- and 3-mm increments) on precision in size structure estimates were as expected; as CL increment increased, fewer crayfish measurements were required to achieve similar precision as indicated by decreasing mean squared differences. Bootstrap analysis indicated little difference between results produced by the 24-sample intensive evaluation data set and the 959-sample data set. The 24-sample data set showed that when CL increment was increased from 1 to 2 mm there were decreases of 4 % and 33 % in overall mean squared differences for crayfish community and the *O. luteus* population size structure estimates, respectively. When CL increment was increased from 1 to 3 mm there were decreases of 60 % and 63 % for crayfish community and *O. luteus* population estimates, respectively. The 959-sample data set showed that when CL increment was increased from 1 to 2 mm there were 33 % and 46 % decreases in mean squared difference for the crayfish community and the *O. luteus* population size structure estimates, respectively. Increasing CL increment from 1 to 3 mm translated to 51 % and 23 % decreases for community and population estimates, respectively.

Results of our attempt to determine feasibility and relative effort required of the relative density method for MDC fisheries management staff were less than desirable. Due to changing

district management priorities, staffing shortages and floods (1993), planned sampling effort by management staff was reduced by 75 % to a total of only 5 days in 3 years.

Based on the limited information, our initial estimates of the number of quadrat samples feasible per day of sampling was slightly high. We expected a team of two biologists working a standard 8-hr day might collect 15 to 20 quadrat samples. Teams averaged 9.3 samples (range = 6 to 12) per day.

Results of the two-mean comparisons on relative density data indicated that power to detect crayfish density differences between any two years was poor in riffle macrohabitats and variable, but generally higher in run macrohabitats (Table 5). For the multiple-mean comparisons we performed to assess the power associated with different sample sizes over a 5-year period, we used sample sizes of 8 and 14 quadrats. We chose to evaluate power at the 8- and 14-quadrat levels of effort because the bootstrapping analysis results (above) showed that taking fewer than 8 samples radically reduced precision, but taking more than 12 or 14 samples did not significantly increase precision. This analysis yielded low power when sampling at the 8-quadrat level in both macrohabitats (Table 6). Power at the 14-quadrat level of effort remained low in riffle macrohabitats, but was high in run macrohabitats. The overall variability among groups of tested mean densities ("effect size") appeared to influence power within a level of effort (i.e. greater effect size contributed to greater power).

Discussion

Trapping

We evaluated the use of baited traps because of their unknown potential for use in

quantitative assessments of stream-dwelling crayfish abundance. They were attractive because they are easy to deploy, minimal training of staff is required, and cost is minimal; they appeared especially advantageous as a potential monitoring tool. Traps have been used extensively in lentic waters. Previous workers (Capelli and Magnuson 1983, Collins et al. 1983, Olsen et al. 1991, Skurdal et al. 1992, Somers and Green 1993) have demonstrated they are effective for obtaining relative abundance estimates in lakes, despite known size, sex and seasonal biases (especially if correction factors are used), and Acosta and Perry (2000) demonstrated their effectiveness for monitoring density in marshes. Conversely, we found almost no information about the effectiveness of traps for quantitative sampling of stream-dwelling crayfish. Brown and Brewis (1978) tested traps in a lotic situation (aqueduct between reservoirs) and recommended them only as an auxilliary method in population studies, for example as a tool for the capture or the recapture portion (but not both) of a mark - recapture study.

We experienced several problems with traps in our stream study, including size and sex biases, high sampling variance and many empty traps, and theft. Size and sex biases would probably lead to overestimation of older age classes and more aggressive crayfish species, and therefore not be conducive to studies of species composition or size class structure in streams (Olsen et al. 1991). The high variance and large number of empty traps would seriously complicate experimental designs and statistical analyses of relative abundance sampling. Our modified (enlarged) trap openings helped address the problem of empty traps but added further to the size bias problem. Stuecheli (1991) suggested that using a variety of trap opening sizes would sample a broader segment of a crayfish population; but it would complicate experimental design and analyses, and would not address other documented biases. During our study we lost several traps to apparent theft, and we believe this would be a continual problem in Ozarks streams with

high recreational use. Another concern about using baited trapping in streams (as opposed to lakes or marshes) was the potential for violating the basic sampling assumption of equal probability of capture (Ricker 1975) and not being able to measure nor estimate the area effectively sampled (Acosta and Perry 2000), because the distribution of the bait odor is subject to currents varying in direction and intensity.

Baited traps were not an acceptable sampling gear for quantitative assessments of stream-dwelling crayfishes. This evaluation was based on a relatively small sample size, but problems and biases emerged so early in the evaluation and were so severe that we quickly eliminated them as a quantitative gear. However, we believe traps have a supplemental role in obtaining qualitative data, particularly in capturing the largest individuals in the crayfish community.

Quadrat sampling

Quantitative sampling method. -Many aspects of research and management of stream-dwelling crayfishes require a reliable and efficient quantitative method, including a gear and a procedure, to sample and evaluate populations and communities. However, there has been limited work to develop such methods (Westman et al. 1978, Rabeni et al. 1997). Several investigators have reported densities of stream-dwelling crayfishes (see reviews in Momot et al. 1978, Hogger 1988, DiStefano 1993), but few of these evaluated the reliability of their sampling method. Our major objectives were to 1) develop a method to obtain representative and precise stream crayfish density estimates that could be used for temporal and spatial comparisons, and 2) to describe general diurnal habitat associations by Ozarks stream crayfish communities. Our 1-m^2 quadrat sampling method was appropriate to address these objectives, but was not without problems.

The 1-m^2 quadrat sampler was an effective gear for sampling crayfish communities in

Jacks Fork and Big Piney rivers. It provided several advantages including a known area for density estimates, and because good visibility was not required it could be used in turbid streams. The high enclosed sides and the bottom flaps that seal into the substrate addressed weaknesses of open-sided known-area gears such as the Surber sampler which have problems with escapement, immigration of organisms from outside the sample area, and are less effective in habitats with minimal current (Bretschko 1990, Brooks 1994). This sampler also had limitations. In deeper waters some crayfish undoubtedly escaped over the top of the sampler despite attempts to restrict it. Our ability to sample the entire crayfish community was hindered because the quadrat was restricted to sampling substrates smaller than 1 m in diameter and could not sample crevices in the limestone bluff walls that are characteristic of many Ozarks streams. Many of the largest crayfish (age 2+ and perhaps 3+) in these streams use these habitats and thus were underestimated in our samples. However, they compose a relatively small part of the crayfish community.

The first step of our quadrat sampling procedure (delineating primary and secondary habitat) was appropriate for both rivers, as suggested by the high classification rates produced in the DFA. It is important to note that our classification rates were optimistically biased because we used the same data to define and evaluate the classification rules (Dillon and Goldstein 1984). Despite the bias, the high classification rates suggest that our stratification method was easily repeatable and precise, allowing us to distinguish primary from secondary habitats.

The second step of our procedure, stratifying quadrat sampling among five macrohabitats, allowed us to partially mitigate the higher variance normally associated with simple random sampling of organisms that exhibit spatial heterogeneity (Morin 1985, Thompson et al. 1992). This facilitated detection of numerous temporal (among years) and spatial (among macrohabitats and sites) differences in crayfish densities. Our sampling precision, as measured by coefficients

of variation, was not high, but acceptable, especially when compared to published values from a similar study by Roell and Orth (1992). Precision was also variable among both spatial and temporal components of the analysis. Precision of means in sampling for benthic invertebrates tends to vary with and is effected by magnitude of the mean, physical size of the sample (quadrat size) and number of samples (Morin 1985, Cooper et al. 1997); only the latter two factors can be controlled and thus modified to improve precision. Prior to the study, we debated the size of our quadrat sampler. A smaller quadrat would have been easier to manipulate, but would have excluded sampling in some larger substrates (particularly large cobble and small boulder) and smaller sampling units typically lead to higher variance (Morin 1985). We believed that a quadrat much larger than 1 m^2 would be unwieldy and impractical. Lamontagne and Rasmussen (1993) required fewer samples with a 10-m^2 quadrat sampler to obtain similar crayfish densities as a 1-m^2 sampler in northern lakes; but the smaller sampler was more efficient at densities above 0.3 • m^{-2} because it required less overall effort. During our study sample sizes were constrained by the number of sampling sites, sampling periods, and personnel. However, in future work we could attempt to increase precision by increasing the overall number of quadrat samples allocated among macrohabitats.

Precision associated with our quadrat sampling method was less than might be desired for some crayfish sampling efforts, but this could be misleading because small coefficients of variation can be produced with highly biased sampling methods (Rabeni et al. 1997). Precision was not the only important variable considered in our evaluation. Future stream crayfish sampling efforts will often be designed to detect potential changes or differences in densities or population levels attributed to pollutants, physical habitat changes, invasive species, or predators. Good statistical power is as important as precision in such sampling efforts (Downing and Downing

1992) as it assesses the ability of a study to detect changes or differences when they truly exist (Somers 1997). A standard criteria for power has not been developed (Somers 1997), but our analyses showed that overall, the quadrat sampling method had good power (78 % or greater in four of five years) to detect spatial differences. Seventy-eight percent power implies a 22 % chance of Type II error, or that about four out of five statistically significant effects were detected in those years. Power to detect temporal changes was generally very low when only 3 years' data were analyzed, but increased to acceptable levels in two of the five macrohabitats when 5 years of data were used, suggesting that we could detect temporal changes among the five macrohabitats if we increased the length of study. Our power was a function of the size of mean crayfish densities, the effect size (or difference among those means), sample size and the alpha level of $P = 0.05$. In future sampling we could increase power to detect spatial or temporal differences by decreasing the amount of error associated with each sample (thereby increasing the relative effect size), increasing the number of quadrat samples allocated among the macrohabitats, or decreasing the alpha level (Somers 1997).

An additional concern with most sampling methods is the potential for operator-induced error. Stream benthic sampling methods have been shown to be susceptible to variation associated with different operators (Pollard 1981), but methods that employ box-type quantitative samplers, like our quadrat, are probably less susceptible than many qualitative methods (Clifford and Casey 1992). We believe we minimized such bias by extensively training field crews and using a standardized procedure.

Quantitative quadrat sampling documented the importance of all five macrohabitats to crayfish communities in Jacks Fork and Big Piney rivers. Macrohabitats with slower current velocities generally had highest crayfish densities. Consistently high crayfish densities in

backwaters and vegetation patches indicated they were important habitats despite their relatively sparse distribution (5 % and 2 % of the stream habitat, respectively; unpublished data). This reinforces the idea that the crayfish communities of these two Ozarks streams are not distributed randomly, but are exhibiting macrohabitat selectivity (Flinders 2000). Several factors probably interact to make these two macrohabitats attractive to a much of the crayfish community, particularly young of year (DiStefano, unpublished data). These factors might include slow current velocities which might allow crayfish to feed more effectively (Hart 1992) and save energy that could otherwise be expended holding station (Maude and Williams 1983), shallow depth for predator avoidance (Blake and Hart 1993), abundance of detritus (in backwaters) for feeding (Whitledge and Rabeni 1997), and presence of macrophytes (in vegetation patches) for predator avoidance (Blake and Hart 1993, Jordan et al. 1996) and their associated epiphytes and insects for feeding (Whitledge and Rabeni 1997).

There are few published studies that have quantitatively documented stream-dwelling crayfish habitat associations or use (Vannote and Ball 1972, Butler and Stein 1985, Rabeni 1985, Roell and Orth 1992, Flinders 2000), but to our knowledge none were as intensive over an extended period of time as this study. Only Flinders (2000) examined crayfish densities in more than three macrohabitats (6), also in two Ozarks streams. Similar to our results, Flinders (2000) reported higher crayfish densities in emergent vegetation and backwaters than in riffles and runs.

Edgewater sampling. -Early observations suggesting higher mean crayfish densities in shallower (< 0.15 m depth) edgewaters were not supported by comparisons between edgewater samples and all other run and pool samples combined. We based this primarily on the overlapping 95 % confidence intervals (Figure 6). However, calculated mean crayfish densities in edgewater

samples were higher at both rivers during both seasons, and 95 % confidence intervals associated with the edgewater means were wider than those around the pool/run means. Our analysis probably suffered from much smaller sample sizes for edgewater data than those for pool/run data. Additionally, in retrospect, it might have been more logical to compare edgewater samples obtained in runs to all other run samples and edgewater samples obtained in pools to all other pool samples. These factors suggest that variance associated with edgewater sampling and our simple analysis masked potential differences.

There is a biological basis to hypothesize that crayfishes, particularly young-of-year, might be attracted to shallow edgewater habitats. These areas were always of depths less than 0.15 m, typically had slow current velocities (< 0.03 m $\bullet$ s^{-1}), and typically contained food items such as algae, detritus and insect larvae (Whitledge and Rabeni 1997, personal observation). Current velocity directly and indirectly influences instream distribution of crayfish (Maude and Williams 1983, Flinders 2000); slower velocities might allow young crayfish to channel less of their energy into holding station and more into growth, or they might result in a more favorable substrate composition. Foraging might also be easier in slower currents (Hart 1992). Shallow depths probably provide young crayfish refugia from most age classes of centrarchid fishes that are among their most important predators (DiStefano unpublished data, Probst et al. 1984, Rabeni 1992, Blake and Hart 1993).

Flinders (2000) included "stream margin" as one of six macrohabitats sampled in a study of stream-dwelling crayfish communities in Ozarks streams. That macrohabitat was essentially the same as our edgewater macrohabitat (Camille Flinders, University of Central Arkansas, personal communication), and in that study, only emergent vegetation consistently harbored higher crayfish densities. Additionally, stream margins were sometimes associated with different species - size

classes than pool or run macrohabitats, supporting it's designation as a distinct macrohabitat (Flinders 2000). We suggest that future studies of Ozarks crayfish communities consider incorporation of an edgewater or stream margin macrohabitat into the study design, particularly in larger streams.

Relative density method. -Our attempt to develop a relative density method to routinely monitor and detect noteworthy temporal changes in stream crayfish communities with reasonable sampling effort (1 day per site each year) was marginally successful. The method was better for estimating relative densities than it was for assessing size structure. Many of the problems encountered could be addressed with increased sampling effort.

Results of the bootstrapping analyses suggested that sampling for more than 1 day at a site would not significantly improve precision for estimating relative densities; but the poor statistical power could be remedied with increased sampling. Precision associated with 1 day's sampling to assess size structure was poor, but could be improved by using larger than 1-mm CL increments.

The third phase of our evaluation suggested that two-person teams conducting routine monitoring might obtain fewer quadrat samples (9) per day than initially expected (15 to 20), and fewer than the number that would be recommended by the first two phases of our evaluation and the power analysis. However, this was based on limited sampling, under less than desirable conditions. Management teams cited inexperience, lack of confidence and equipment malfunctions to explain the lower than estimated productivity (Andrew Austin and Donna Menown, MDC, personal communication). We have determined from 9 years of research using the quadrat method and well-trained, experienced two-person teams, that about 30 min are required (depending upon number of crayfish captured) to obtain and process a quadrat sample in a riffle or run. Therefore,

it should be feasible to obtain 12 to 14 quadrat samples per 8-hr work day.

This method has inherent weaknesses that could not be addressed with increased precision or statistical power. Our design called for sampling only two macrohabitats to reduce required sampling time and eliminate the need for snorkeling or SCUBA equipment, but this introduced bias. Unpublished data (DiStefano) indicated that these 3 crayfish species and their age classes use the five macrohabitats differentially, so segments of the community that were associated with macrohabitats other than riffles or runs would be underestimated in sampling. Such bias may or may not impair a monitoring effort, but must be considered by potential users of this method.

In summary, the relative density method produced unacceptable results under the circumstances we selected; however, it could be easily modified to improve performance and reliability for monitoring depending upon objectives. It is critical to identify *apriori* the objectives of a monitoring program, especially with regard to ability (i.e. statistical power) to detect temporal community or population changes, and then design the sampling program accordingly. If the goal of a program is to monitor aquatic biota under a scenario of perceived environmental threats, it would be prudent to design the program with high statistical power (beta), probably higher than or equal to the alpha level to ensure detection of those effects (Downing and Downing 1992, Somers 1997). However, if a program's goal is only to detect large population or community fluctuations, then lower statistical power (e.g. 80 %) might be acceptable. Relative abundance methods have been used successfully to monitor crayfish populations in lakes (Somers et al. 1996, David et al. 1997), but we found no published work describing methods for monitoring stream-dwelling crayfish populations or communities. We consider our evaluation to be a first step in the development process. However, our primary recommendation to potential users of this method would be to consider increased sampling effort, starting with allocating at least 1

additional day to allow doubling the number of quadrat samples obtained.

Conclusions

Our initial goal was to develop quantitative sampling methods that would help us assess the status of stream-dwelling crayfish communities. We tested two sampling gears and three methods to address that need. Although baited traps have been used successfully in lentic waters to produce relative crayfish abundance estimates, they were unreliable for quantitative assessments in Jacks Fork River. Traps effectively captured crayfish, but results were biased and highly variable. It is conceivable that traps might be used to supplement quantitative data obtained with more reliable methods, especially if there is a need to capture the largest members of the crayfish community.

The 1-m^2 quadrat sampler proved an effective gear for sampling most of the crayfish community in these streams during summer and fall, and the quantitative sampling method performed well in estimating crayfish densities in the 5 macrohabitats and detecting spatial density differences among them. The quadrat proved ineffective in spring due to the timing of hatching in these *Orconectes* species. It is important to incorporate life history events and their potential effects on availability or susceptibility of biota to sampling gears when planning a study, and many invertebrate community studies ignore such considerations (Malley and Reynolds 1979). This method was developed and tailored specifically for use in small to medium sized Ozarks streams. Our intent was not to develop nor prescribe a method with applicability beyond this region, nor to necessarily provide crayfish density data for comparison with other communities. However, given the relative absence of published, reliable methods for quantitatively sampling stream-dwelling

crayfish populations or communities, this method, or parts of it, may prove valuable to workers in other regions. Downing and Downing (1992) reported that 85 % of published density estimates of benthic invertebrates were based on only three or fewer samples. Our method is somewhat more labor and time intensive, but it reduces a substantial amount of variability and provides more confidence in density estimates than many other stream crayfish sampling methods. Future efforts could include modifications to improve performance detecting temporal changes in crayfish densities and possibly to effectively sample all segments of the community. Such modifications might include increasing statistical power by increasing sample size, or incorporating a second sampling gear or technique (Malley and Reynolds 1979), to obtain quantitative data on the oldest age class(es), although we believe that segment of the crayfish community in these Ozarks streams is very small.

The relative density method we evaluated showed some promise for monitoring and assessment of significant crayfish community fluctuations, but would require additional modification and testing. This method was intended to be less intensive in terms of time and effort so that it might be more attractive to stream managers. However, results showed that meaningful data collection might require more than 1 day of sampling per site. Additionally, sampling in only riffles and runs could lead to underestimation of populations that typically associate with slower velocity, deeper macrohabitats.

It is uncommon for aquatic biologists to have the opportunity to allocate several years and significant resources toward evaluating sampling gears and methods. Despite that luxury, the methods we developed were not without problems. Throughout this study, it was apparent that no single sampling gear or method was appropriate for every situation, even within the same water body. In addition, our experience reaffirmed that quantitative community studies should

incorporate pilot studies and sufficient time for evaluation of sampling gears and methods.

The 1-m^2 quadrat sampling method demonstrated that although Jacks Fork and Big Piney rivers are distinctly different Ozarks streams, their crayfish communities were similar in that they used a variety of habitats, and they tended to use them in a similar pattern. These patterns remained consistent over several years, but densities within distinct macrohabitat types showed significant interannual variation. Macrohabitats featuring emergent vegetation patches and shallow backwater pools consistently harbored the highest diurnal mean crayfish densities, whereas riffles held the lowest crayfish numbers. Future work will employ the 1-m^2 quadrat sampling method to study how crayfish species in these communities partitioned available habitats, and to examine how crayfish production was allocated among the macrohabitats.

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TABLE 1. Means and ranges (in parentheses) of selected water chemistry parameters measured at Jacks Fork River (JF) and Big Piney River (BP) study sites[a] during 1994 through 1999. Measurements were made at approximately 0900 h at each site, May through September.

River	Site	Dissolved O2 (mg/l)	pH	Total NH3 (mg/l)	Alkalinity (mg/l as CaCO3)	Hardness (mg/l as CaCO3)	Conductivity µmhos
JF	1	9.2	8.19	0.053	175	216	336
N = 15		(7.0 - 10.9)	(8.05 - 8.45)	(0.016 - 0.094)	(135 - 217)	(125 - 380)	(201 - 390)
JF	2	9.1	8.12	0.058	176	210	345
N = 13		(7.0 - 10.9)	(7.81 - 8.39)	(0.014 - 0.088)	(130 - 223)	(122 - 330)	(305- 408)
BP	1	9.0	8.02	0.064	171	197	359
N = 20		(5.8 - 10.7)	(7.03 - 8.71)	(0.010 - 0.260)	(101 - 243)	(140 - 310)	(160 - 515)
BP	2	8.5	8.01	0.066	160	197	345
N = 15		(6.0 - 12.0)	(7.78 - 9.23)	(0.013 - 0.170)	(130 - 211)	(98 - 340)	(248 - 410)

[a] Jacks Fork River sites were located at Ratcliff Ford (Site 1, 1.3 km in length) and Blue Spring (Site 2, 1.2 km), and Big Piney River sites were located at Mineral Spring (Site 1, 0.8 km) and Sand Shoals (Site 2, 1.3 km).

TABLE 2. Results of two-way ANOVAs (summer data), three-way ANOVAs (fall data) and LSMPDAs comparing crayfish mean densities among macrohabitats in Jacks Fork and Big Piney rivers, 1991 - 1998.

	ANOVA statistics			
	F	df	MSE	P
Jacks Fork - summer[a]	20.15	4, 300	0.5024	< 0.0001
Jacks Fork - fall[b]	32.55	4, 361	0.3403	< 0.0001
Big Piney - summer[c]	45.88	4, 288	0.4061	< 0.0001
Big Piney - fall[d]	2.68	2, 144	0.4054	0.0721

[a]Jacks Fork River, year x macrohabitat interaction was not significant (P = 0.6081).

[b]Jacks Fork River, year x site x macrohabitat interaction was significant (P = 0.0049), but ordered (Ott 1988). LSMPDA showed vegetation patches differed from riffles (P < 0.0001) and runs (P < 0.0001); riffles differed from backwater/forewater pools (P < 0.0001) and pools (P < 0.0001).

[c]Big Piney River, year x macrohabitat interaction was significant (P = 0.0330) but ordered (Ott 1988). LSMPDA showed vegetation patches and backwater/forewater pools differed from all other macrohabitats (all comparisons, P < 0.0001); pools differed from riffles (P = 0.0084).

[d]The analyses for Big Piney River fall sampling included only the macrohabitats riffles, runs and pools; all data for backwaters and vegetation patches were excluded due to a lack of those samples in 1993.

TABLE 3. Results of an analysis to determine statistical power of comparisons of mean crayfish densities (number • m^{-2}) using 5 years of data form Jacks Fork River, Missouri. Column on far right represents statistical power of comparisons within a macrohabitat across years; row on bottom represents power of comparisons within years across macrohabitats.

	Mean density						
Habitat	1994	1995	1996	1997	1998	P-value	Power
Riffle	20.5	11.4	13.1	7.4	10.9	0.0393	0.43
Run	34.7	27.8	21.3	10.4	14.0	0.0001	0.97
Pool	42.9	37.9	26.6	21.0	24.6	0.0021	0.85
Vegetation	43.4	40.0	30.0	23.0	35.0	0.3889	0.00[a]
Backwater	45.8	55.8	38.4	28.6	17.4	0.2557	<0.38
P-value	.2760	.0001	.0004	.0064	.0041		
Power	<0.38	.99	.96	.78	.80		

[a] Power value of 0.00 achieved when the mean square of the error term equals the mean square of the model term (Somers 1997).

TABLE 4. Results of an analysis to determine statistical power of comparisons of mean crayfish densities (number • m^{-2}) using 3 years of data form Jacks Fork River, Missouri. Column on far right represents statistical power of comparisons within a macrohabitat across years.

	Mean density				
Habitat	1994	1996	1998	P-value	Power
Riffle	17.1	12.6	9.3	0.0677	0.40
Run	26.9	19.0	13.8	0.2314	0.20
Pool	34.5	26.8	23.4	0.1326	0.30
Vegetation	35.6	30.5	33.3	0.7702	N/A[a]
Backwater	35.0	31.4	23.9	0.3595	0.00[b]

[a] Mean square of error term was greater than the mean square of the model term, producing a negative phi value (Somers 1997).

[b] Mean square of error term was equal to the mean square of the model term (Somers 1997).

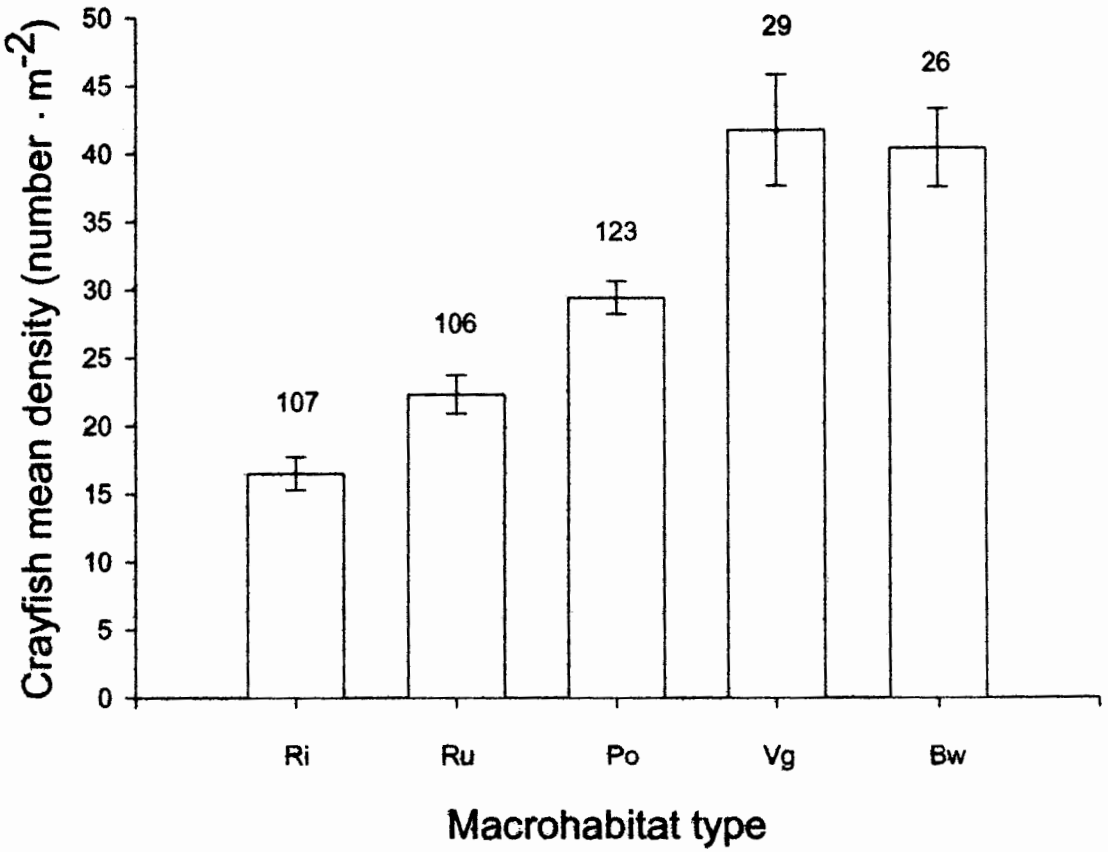

FIG. 3. Comparison of crayfish mean densities (number $\cdot m^{-2}$), all three species and ages combined, among five macrohabitat types (Ri = riffles, Ru = runs, Po = pools, Vg = emergent vegetation patches, Bw = backwater/forewater pools) at Jacks Fork River during fall 1991 through 1993. Error bars represent ± 1 standard error. Sample sizes (number of 1-m^2 quadrats, N) appear above bars. Densities in vegetation patches were significantly ($P < 0.05$) higher than those in riffles and runs across all years. Densities in pools and backwater/forewater pools were significantly higher than those in riffles across all years.

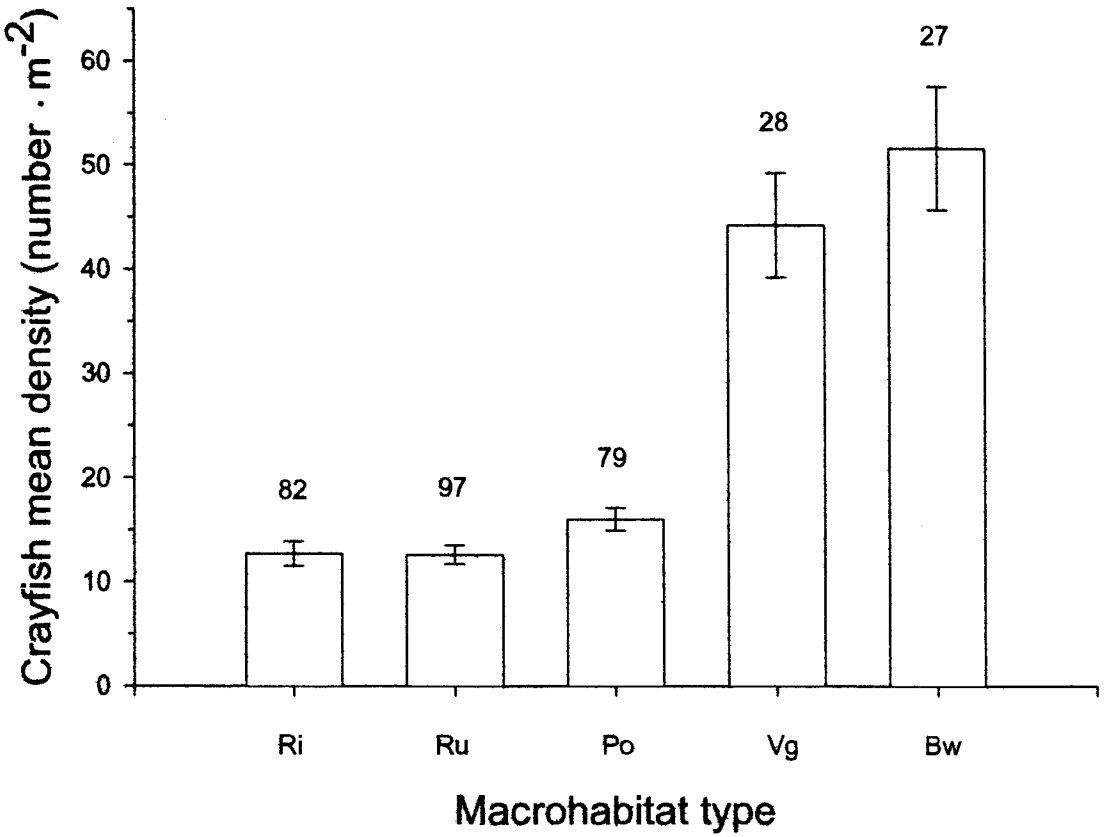

FIG. 4. Comparison of crayfish mean densities (number · m^{-2}), both species and ages combined, among five macrohabitat types (Ri = riffles, Ru = runs, Po = pools, Vg = emergent vegetation patches, Bw = backwater/forewater pools) at Big Piney River (site 2 only) during summer 1994 through 1998. Error bars represent ±1 standard error. Sample sizes (number of 1-m^2 quadrats, N) appear above bars. Densities in vegetation patches and backwater/forewater pools were significantly ($P < 0.05$) higher than those in all other macrohabitats across all years. Densities in pools were significantly higher than those in riffles across all years.

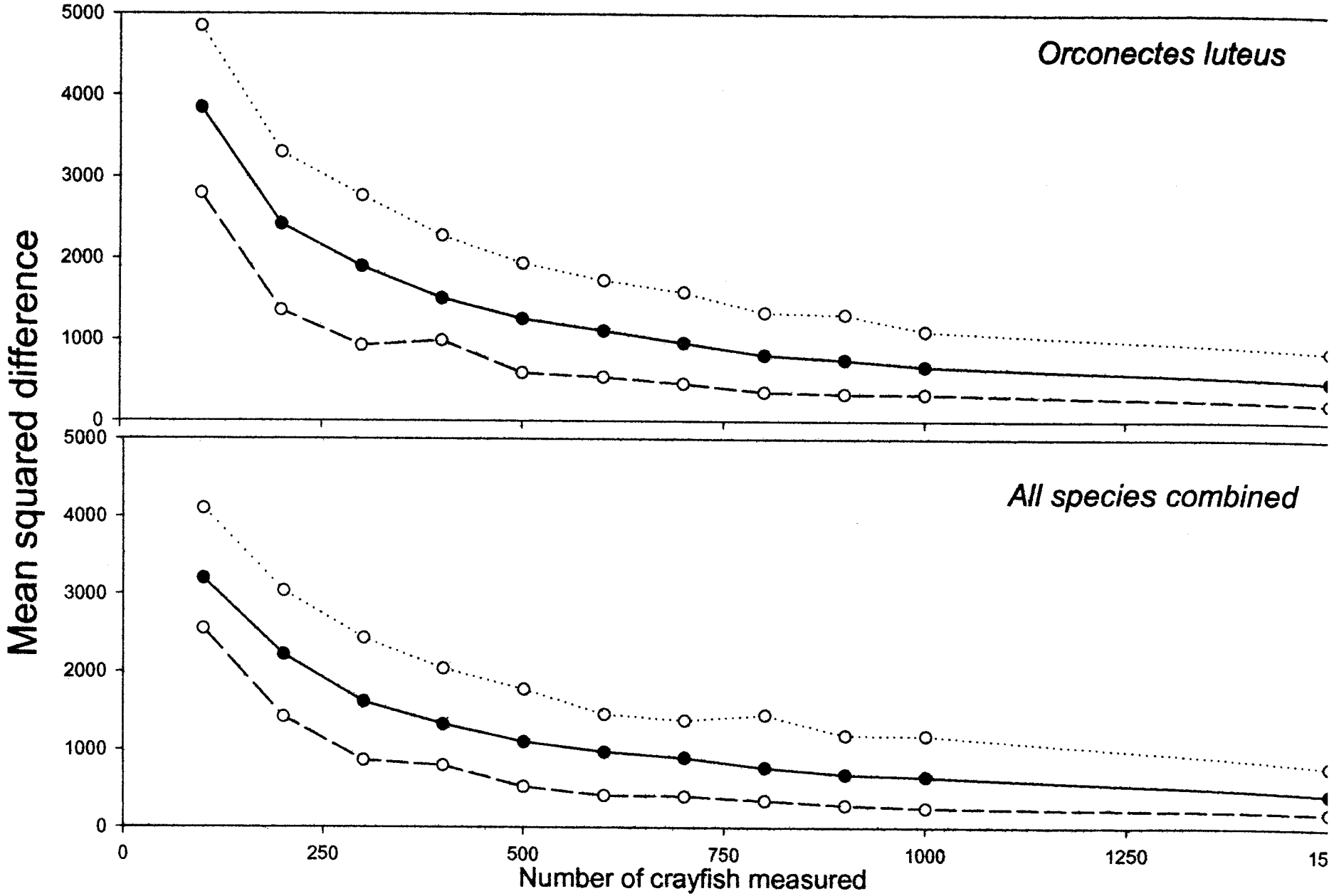

FIG. 9. Results of a bootstrapping technique used in the first phase of an evaluation of a potential method to estimate relative crayfish size structures in Jacks Fork River, Missouri. Solid circles represent mean squared differences for a given number of crayfish carapace lengths (1-mm increments) obtained in riffle and run macrohabitats on July 21, 1993. Open circles represent 90 % confidence intervals. Estimates are based on repeated random sampling (with replacement) of a 24-sample data set. Results for the *Orconectes luteus* population are on top; results for the crayfish community are on the bottom.

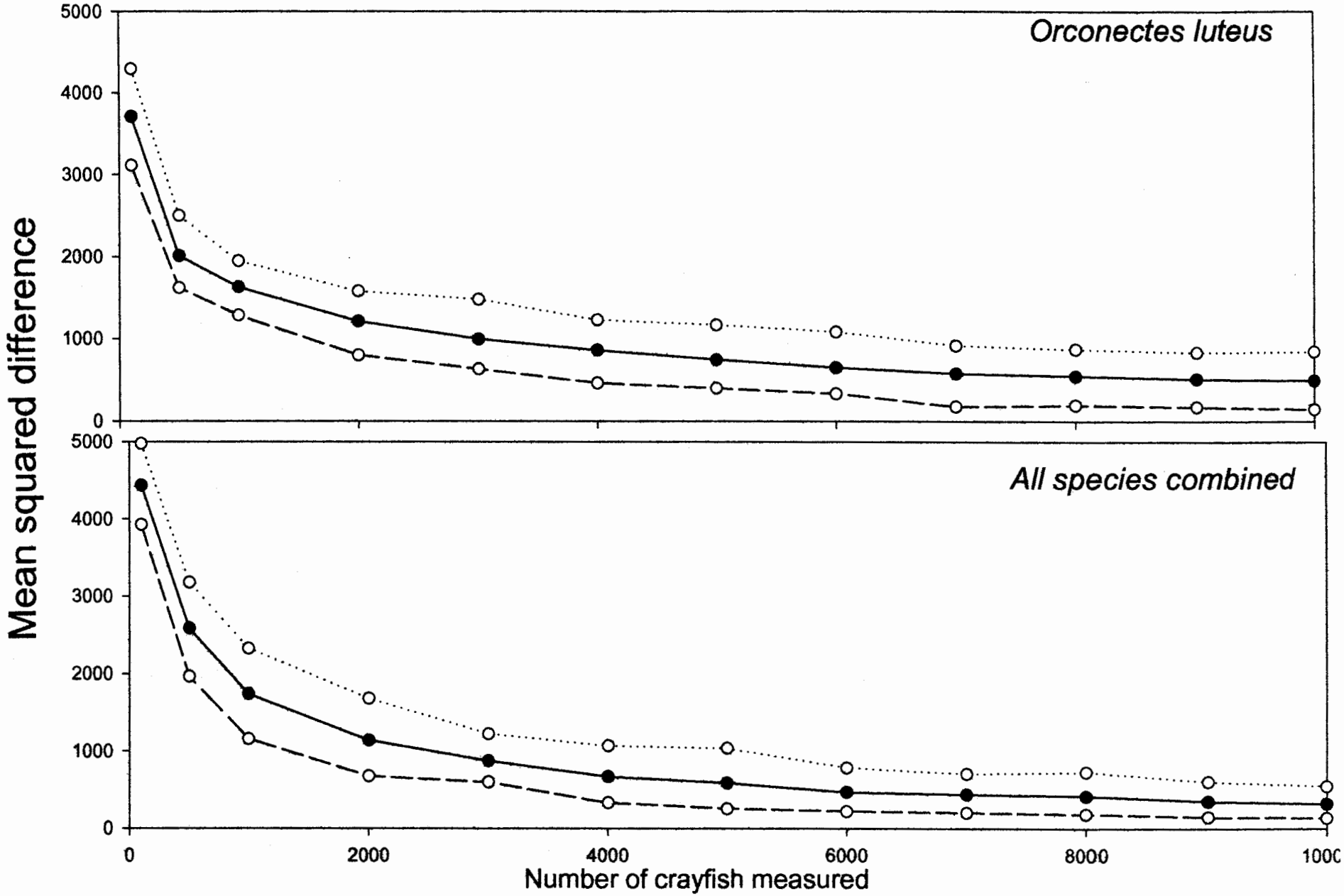

FIG. 10. Results of a bootstrapping technique used in the second phase of an evaluation of a potential method to estimate relative crayfish size structures in Jacks Fork River, Missouri. Solid circles represent mean squared differences for a given number of crayfish carapace lengths (1-mm increments) obtained in riffle and run macrohabitats from 1991 through 1998. Open circles represent 90 % confidence intervals. Estimates are based on repeated random sampling (with replacement) of a 959-sample data set. *Orconectes luteus* population are on top; results for the crayfish community are on the bottom.

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